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Novel Method Reveals a Narrow Phylogenetic Distribution of Bacterial Dispersers in Environmental Communities Exposed to Low-Hydration Conditions

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ABSTRACT In this study, we developed a method that provides profiles of community-level surface dispersal from environmental samples under controlled hydration conditions and enables us to isolate and uncover the diversity of the fastest bacterial dispersers. The method expands on the porous surface model (PSM), previously used to monitor the dispersal of individual bacterial strains in liquid films at the surface of a porous ceramic disc. The novel procedure targets complex communities and captures the dispersed bacteria on a solid medium for growth and detection. The method was first validated by distinguishing motile *Pseudomonas putida* and *Flavobacterium johnsoniae* strains from their nonmotile mutants. Applying the method to soil and lake water bacterial communities showed that community-scale dispersal declined as conditions became drier. However, for both communities, dispersal was detected even under low-hydration conditions (matric potential, -3.1 kPa) previously proven too dry for *P. putida* strain KT2440 motility. We were then able to specifically recover and characterize the fastest dispersers from the inoculated communities. For both soil and lake samples, 16S rRNA gene amplicon sequencing revealed that the fastest dispersers were substantially less diverse than the total communities. The dispersing fraction of the soil microbial community was dominated by *Pseudomonas* species cells, which increased in abundance under low-hydration conditions, while the dispersing fraction of the lake community was dominated by *Aeromonas* species cells and, under wet conditions (-0.5 kPa), also by *Exiguobacterium* species cells. The results gained in this study bring us a step closer to assessing the dispersal ability within complex communities under environmentally relevant conditions.

IMPORTANCE Dispersal is a key process of bacterial community assembly, and yet, very few attempts have been made to assess bacterial dispersal at the community level, as the focus has previously been on pure-culture studies. A crucial factor for dispersal in habitats where hydration conditions vary, such as soils, is the thickness of the liquid films surrounding solid surfaces, but little is known about how the ability to disperse in such films varies within bacterial communities. Therefore, we developed a method to profile community dispersal and identify fast dispersers on a rough surface resembling soil surfaces. Our results suggest that within the motile fraction of a bacterial community, only a minority of the bacterial types are able to disperse in the thinnest liquid films. During dry periods, these efficient dispersers can gain a significant fitness advantage through their ability to colonize new habitats ahead of the rest of the community.

KEYWORDS community motility, *Pseudomonas putida* KT2440, liquid film, soil, lake water, succession, porous surface model

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Dispersal is essential in order to ensure fitness in a world of limited and heterogeneously distributed resources and is recognized as a key contributor to community dynamics (1, 2). While dispersal has long been studied as an integral part of the ecology of animals and plants, its contribution to microbial ecology has received less attention (3–5).

Dispersal is traditionally divided into passive modes (caused, e.g., by weather or human activities) and active modes, also termed motility, which requires metabolic energy (6). Motility is not limited to environments saturated with water but is also commonly found on or near surfaces in unsaturated environments, such as the thin liquid films between soil particles or on the surface of leaves (7). Bacteria have evolved diverse mechanisms of active dispersal on surfaces, including swimming, swarming, twitching, sliding, and gliding, all of which have been mainly described and studied in pure-culture settings (8–11), using agar plates or glass slides for the study of motile cells (8, 9, 11, 12). Hence, it remains unclear how well these methods capture dispersal potential in more natural settings, such as soils, and how the ability to disperse is distributed within the tree of life and within individual communities. There have been a few efforts to uncover the phylogenetic distribution of flagellar motility (13, 14) and gliding motility (12, 15), but a comprehensive view of how the dispersal abilities vary across and within bacterial phyla is still lacking.

This gap in our knowledge results partly from the lack of methods for mass assessment of the dispersal potential of bacteria in environmental samples. In a community, not all bacteria have equal potentials for dispersal, though this is rarely assessed. To assess the dispersal potential of a community, one could, in theory, isolate and test all its members, but considering that there can be up to 10^9 bacterial cells in a gram of productive soil (16), this would be unfeasible in practical terms. In addition, by studying strains in isolation, the effects of interaction between strains would be missed. Indeed, most past studies of motility have focused on the motion of single strains (8, 17, 18), largely neglecting the vast possibilities of interactions which have only recently been uncovered (17). Bacterial cocultures have been observed swarming together, combining their abilities to conquer barriers like antibiotics (19) or to engage in metabolic cross-feeding (20). Motile bacteria have been demonstrated to carry nonmotile bacteria as cargo (21), and interkingdom cooperation has been described, such as bacterial dispersal with the help of fungi and amoebas (22–26). It would seem logical that these complex interactions occur in natural communities, but only a very few attempts have been made to tackle motile bacteria in environmental samples at the community level (27–31).

A few studies did address community-level motility in aquatic environments. Grosart et al. (29), Mitchell et al. (31), and Fenchel (30) assessed swimming motility in ocean samples using microscopy and revealed large percentages of motile bacteria, but did not identify them. Dennis et al. (27) used a syringe-based assay and 16S rRNA gene amplicon sequencing to uncover the identities of motile lake water bacteria, showing a chemotactic response toward inorganic substrates. However, to our knowledge, only one study assessed the dispersal and identities of dispersing bacteria in a complex natural community under conditions relevant for partially water-saturated habitats (e.g., surface or vadose zone soils; the phyllosphere) (28). Using sand microcosms, Wolf et al. revealed that a subset of a soil community consisting mainly of members of the family *Enterobacteriaceae* and the genera *Undibacterium*, *Pseudomonas*, and *Massilia* were able to expand to a distance of more than 2 cm from the inoculation point within 48 h (28). While that study provided important insights into the identities of dispersers and their expansion rate, the authors only considered one hydration condition (7.5% moisture [wt/wt], i.e., a matric potential in the -20 - to -50 -kPa range based on the particle size [32]), yet previous studies have stated that water is one of the primary factors controlling bacterial motility (33).

In a nonpermanently water-saturated habitat like soil, the ability to disperse is primarily dependent on the thickness of the water film surrounding solid surfaces. The hydration status of soil in the vadose zone is highly variable and can increase or

decrease rapidly, e.g., following rainfall or drought (7, 18, 33). At low matric potential, the thinning of the aqueous films between soil particles will lead to habitat fragmentation into separate microhabitats (34), with strong effects on bacterial dispersal abilities.

The porous surface model (PSM) is a 2-dimensional model system used for studying bacterial motility on the surface of a porous ceramic disc under controlled hydration conditions, which mimics unsaturated soil surfaces. Studies of fluorescently tagged pure cultures using the PSM have demonstrated that flagellar motility is restricted to a relatively narrow range of water potential (0 to -2 kPa) (18, 34). However, it remains unclear how this knowledge about specific flagellated bacterial isolates can be translated at the scale of complex environmental communities.

Hence, the aims of the current study were to (i) further develop the PSM for its use in assessing bacterial dispersal of natural (untagged) bacterial communities and (ii) apply the method to a soil and a lake community to obtain community-level surface motility profiles under controlled hydration conditions and uncover the diversity of the fastest dispersers.

RESULTS

Developing and validating the novel method with pure cultures. The porous surface model (PSM) has previously been used to monitor bacterial dispersal under controlled hydration conditions (35). The challenge of the current study was to expand the method from being solely usable with fluorescently tagged cultures to be usable to evaluate the dispersal of a broader range of complex natural communities, tracking the movement from the inoculation point at the center toward the edge of the ceramic disc. To achieve this goal, we devised a procedure to get an imprint of dispersal on the ceramic disc by pressing agar plates onto the PSM surface (see Fig. S1 in the supplemental material for an overview of the method). This step resembles the agar lift method used for visualization of the bacterial distribution on soil surfaces described in a previous study (36).

Initial tests with green fluorescent protein (GFP)-labeled *Pseudomonas putida* strain KT2440 (*P. putida* KT2440 GFP) and full agar plates pressed onto the ceramic disc of the PSM showed a clear discrepancy between the bacterial spatial distribution observed on the ceramic disc with epifluorescence microscopy before pressing and that captured on the agar plate. It was clear that pressing the agar plate onto the surface of the ceramic disc disturbed the bacterial distribution, so that the cells present at high density at the inoculation point were often spread over a much larger area. To avoid this error, we developed a series of concentric annular agar plates, which were pressed onto the disc sequentially, starting from the edge of the ceramic disc and leaving the center undisturbed until a full plate was used to capture the total community that had developed on the PSM disc (referred to as the “full plate” hereinafter) (Fig. 1; Fig. S1).

To test the method, we inoculated a mixture of the motile strain *P. putida* KT2440 GFP and its nonflagellated, red fluorescent protein DsRed-labeled mutant *P. putida* KT2440 DsRed $\Delta fliM$, which had previously been used for motility studies on the PSM (18, 35). This pure-culture experiment demonstrated the ability of the method to clearly contrast the dispersal potential of these strains (Fig. 1 and 2). The nonmotile strain generally stayed near the inoculation point. For the motile strain, the fastest dispersal was seen at -0.5 kPa, with bacteria in 4 of 6 replicates reaching the edge of the pressed plate furthest from the inoculation point, i.e., the 25- to 41.3-mm section, after 40 h of incubation (Fig. 2). Dispersal at -1.2 kPa was not significantly different from that at -0.5 kPa after 24 h ($P = 0.229$) or 40 h ($P = 0.857$), and no dispersal was registered at -3.1 kPa. This was consistent with the threshold for flagellar motility of -2.0 kPa previously reported for *P. putida* KT2440 (18).

To test the ability of the method to capture other types of motility than flagellum-powered swimming, we inoculated *Flavobacterium johnsoniae* strain CJ1827 gliding bacteria (37) and nonmotile mutant *F. johnsoniae* strain 2122 $\Delta gldK$ bacteria (38) onto separate PSMs incubated at -0.5 kPa. After 48 h of incubation, the nonmotile mutant bacteria ($n = 2$) stayed near the inoculation point, while the gliding bacteria ($n = 3$)

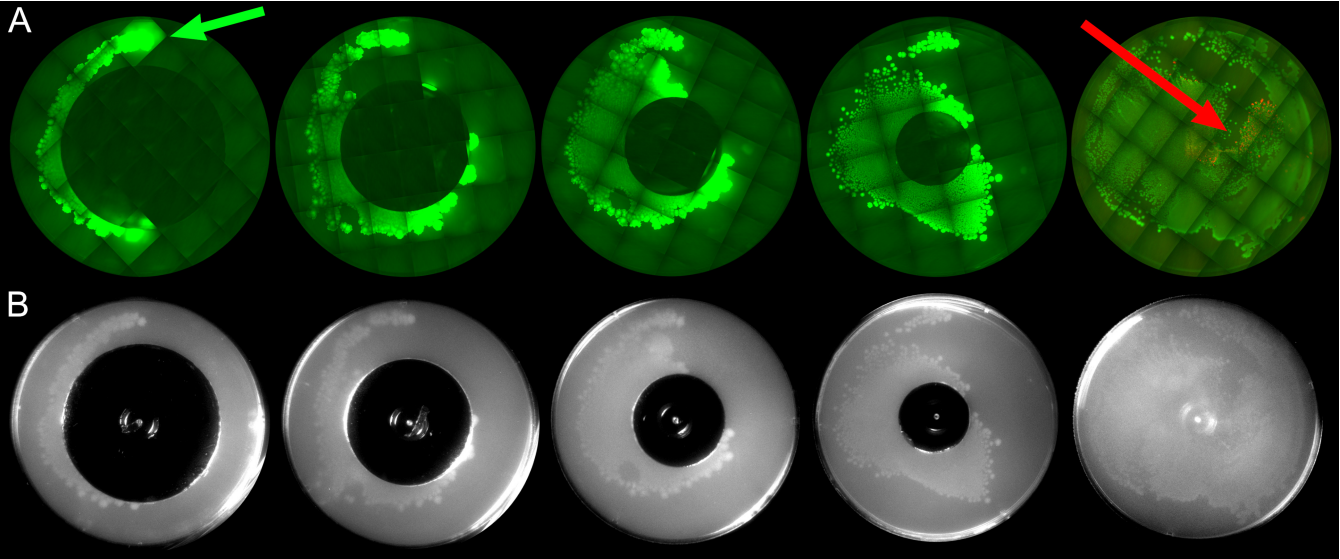


FIG 1 Proof of concept using pure cultures. (A) Separation of the motile strain *P. putida* KT2440 GFP (green) and the nonmotile strain *P. putida* KT2440 DsRed $\Delta fliM$ (red) on agar plates pressed onto the ceramic disc as pictured with multiple fields of epifluorescence microscopy. The nonmotile strain was only detected on the full plate press (red arrow), while the motile strain was detected on all of the pressed plates, including the one that captures the zone most distant from the inoculation point (green arrow). (B) Dispersal assessed with a camera without fluorescence detection, which is the method used for environmental communities. Contrast has been digitally enhanced. The plates were pressed after 40 h of dispersal at -0.5 kPa.

were recovered in the 11.5- to 15-mm section and, in one case, at the edge of the pressed plate, the 25- to 41.3-mm section.

Dispersal potential of environmental communities. Applying the novel method to extracted soil and lake bacterial communities confirmed that dispersal rates declined as

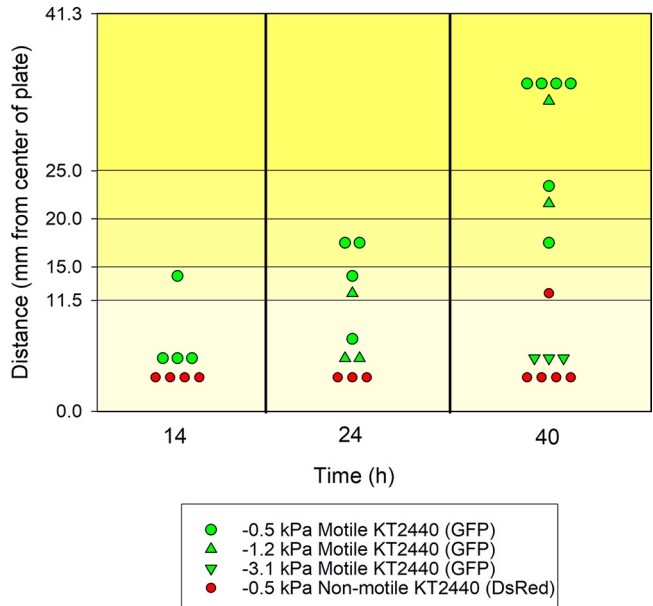


FIG 2 Dispersal dynamics of motile and nonmotile strains as affected by the matric potential. The progressive dispersal of the motile strain *P. putida* KT2440 GFP was captured by our method, as well as the inability of the nonmotile *P. putida* KT2440 $\Delta fliM$ DsRed to disperse away from the center of the ceramic disc. Both the motile (green) and nonmotile (red) strain were tested at three matric potentials. For the nonmotile strain, only the results for -0.5 kPa are depicted, as the other values were similar, with bacteria present solely at the center. The distances shown are ranges; e.g., colonies were observed on the agar ring at distances between 11.5 and 15 mm from the center. The number of replicate dispersal experiments varied from 2 to 5.

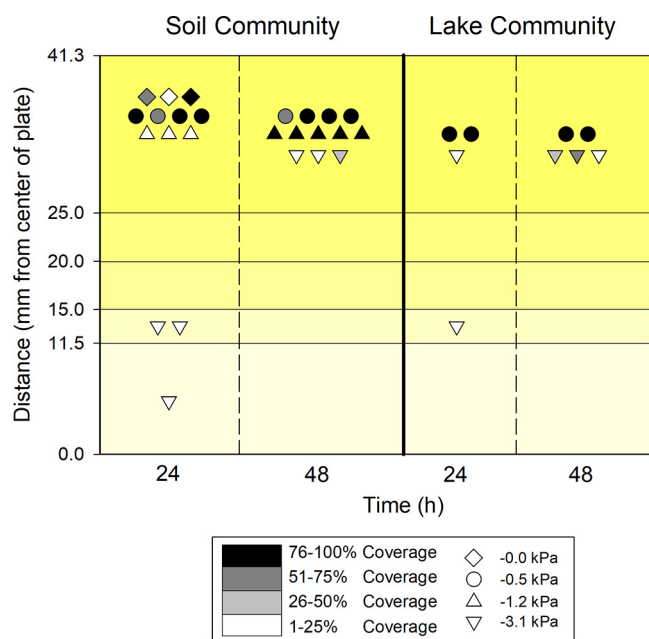


FIG 3 Dispersal of a soil and a lake community over time at different matric potentials. Symbol shading depicts bacterial coverage of the pressed agar plate, giving an indication of the extent of colonization. The lake community was tested at two matric potentials, versus four for the soil one; the number of replicates varied from two to five.

conditions became drier (Fig. 3). However, surprisingly, dispersal of both community types was detected even under the lowest-hydration conditions tested (-3.1 kPa, as well as one sample at -4.2 kPa [not shown]), previously proven too dry for *P. putida* KT2440 dispersal (Fig. 2). For the soil community, the slowest dispersal was detected at -3.1 kPa, with cells in 2 of 3 replicates dispersed to the 11.5- to 15-mm section at 24 h, though cells in all replicates reached the most distant section of the plate (25- to 41.3-mm section) after 48 h (Fig. 3). To record the magnitude of the colonization of the ceramic plate sections, a coverage score was introduced for the environmental samples, where the extent of colony coverage of the agar plates was roughly assigned into four categories. This scoring indicated that even though soil bacteria reached the most distant sections of the plates after 48 h, the colonization was less at -3.1 kPa (1 to 25% to 26 to 50% coverage) than at -1.2 kPa and -0.5 kPa (51 to 76% to 76 to 100% coverage, respectively).

The lake bacterial community study was included mainly for comparison purposes, as we assumed that this community would have experienced a weaker selection for dispersal ability on dry surfaces, and to demonstrate the versatility of the method. It was based on fewer replicates, which limits the interpretation, but revealed a similar picture, with cells reaching the edge of the plate after 48 h under both dry (-3.1 kPa) and wet (-0.5 kPa) conditions, although the extent of colonization was less for the dry samples (Fig. 3). Direct comparisons of the soil and lake data should be done with caution, because the CFU counts suggested that more cultivable cells were inoculated for the lake than for the soil samples (34×10^3 versus 2×10^3 to 8×10^3 CFU per inoculum, respectively). However, for both soil and lake communities, it remains clear that we registered much faster dispersal at both -0.5 kPa and -3.1 kPa (Fig. 3) than for *P. putida* KT2440 cells (Fig. 2).

Diversity of dispersers. DNA was extracted from the Nycodenz soil extracts and the lake filtrate used for inoculations, from agar plates reflecting the total community present on the ceramic disc (full plate community), and from the community that developed upon inoculation of the environmental cell extracts onto a standard 25% R2A solid medium plate with 20 g agar liter $^{-1}$, which provides conditions that are not conducive to motility (39) (the no-motility reference plate, referred to as the “reference

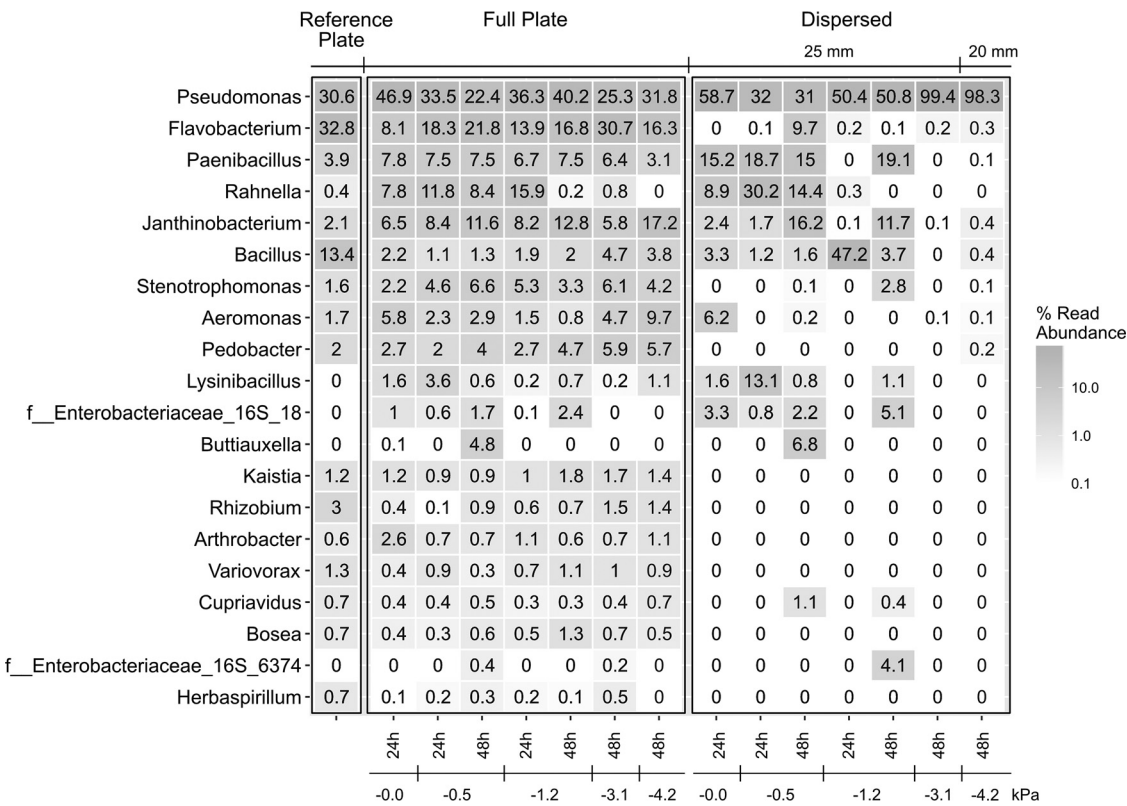


FIG 4 Heatmap of the relative abundances of the 20 most dominant genera across communities derived from a soil extract and differing in their dispersal after being incubated at different prescribed matric potentials for 24 h or 48 h. For 24 h, the results for two additional matric potentials of 0.0 kPa and -4.2 kPa (only one sample recovered at 20 mm) were added. Columns present the average results for triplicate communities except for the restricted-motility control (reference plate; $n = 4$), the total community on the full plate at -4.2 kPa ($n = 1$), and the fastest-dispersed communities at -1.2 kPa for 24 h ($n = 2$) and at -3.1 kPa ($n = 2$) and -4.2 kPa ($n = 1$) for 48 h.

plate” hereinafter). All samples were sequenced using Illumina sequencing targeting the V3-V4 regions of the 16S rRNA gene. A total of 3.8 million sequences were kept after filtering for further analysis.

Comparisons between the communities in the inoculum (Nycodenz extractions and the lake filtrate) and the cultivable communities, dispersed or not, confirm the expected cultivation bias (Fig. S7 and S8). However, the cultivable communities represented on the full plates, dispersed communities, and reference plates retained high diversity, with representatives of 96 unique genera for soil and 86 for lake communities. In addition, 546 and 428 amplicon sequence variants (ASVs) (40) in soil and lake samples were not identifiable at the genus level. Moreover, the dominating genera in the cultivated soil communities (*Pseudomonas*, *Flavobacterium*, and *Paenibacillus*) were also among the abundant taxa of the Nycodenz extractions (Fig. S7) and lake water filtrate (*Aeromonas*, *Flavobacterium*, and *Exiguobacterium*) (Fig. S8).

For each PSM, we collected the DNA of the fastest dispersers, i.e., that of the colonies of the pressed agar plate the furthest from the point of inoculation that presented growth. As the method did not allow for selective recovery of the cells that were unable to disperse, we compared these dispersed communities to the total communities present on the full plate and the reference plate. Sequencing results from the soil community plates showed a dominance of *Pseudomonas* cells in the dispersed communities, benefitting from increasingly dry conditions and achieving almost total dominance under the driest conditions (-3.1 kPa and -4.2 kPa; 99.4 to 98.3% after 48 h) (Fig. 4). Under wet conditions (0.0 kPa and -0.5 kPa), the dispersed bacterial community consisted mainly of *Paenibacillus*, *Rahnella*, and *Lysinibacillus* cells, in addition to *Pseudomonas* cells, and after 48 h (at -0.5 kPa), also of *Flavobacterium* and

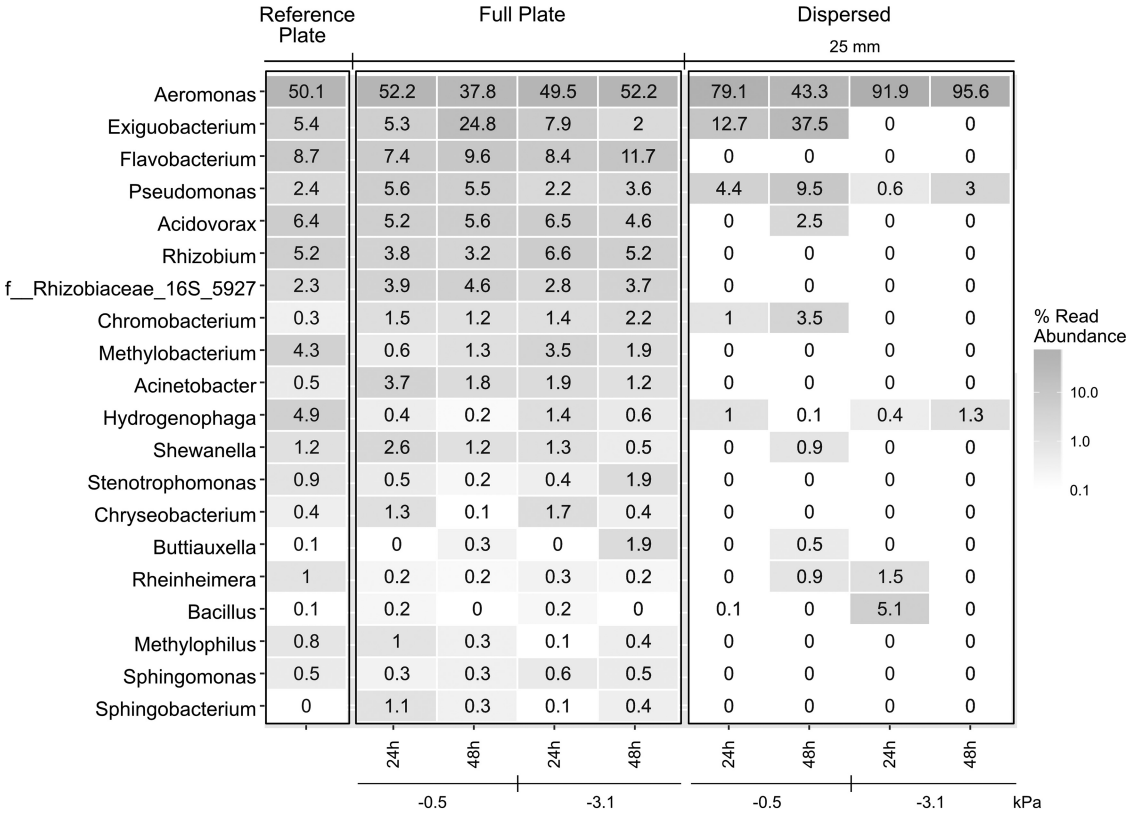


FIG 5 Heatmap of the relative abundances of the 20 most dominant genera across communities derived from a lake filtrate and differing in their dispersal after being incubated at different prescribed matric potentials for 24 h or 48 h. Columns present the average results for duplicate communities, except for the restricted-motility control (reference plate, $n = 1$), the total community on the full plate at -3.1 kPa ($n = 3$) for 48 h, and the fastest-dispersed community at -3.1 kPa for 24 h ($n = 1$).

Janthinobacterium cells. Under conditions of moderate dryness (-1.2 kPa), *Bacillus* cells were almost equal in abundance to *Pseudomonas* cells (47.2% and 50.4%, respectively) at 24 h but were reduced over time to 3.7% at 48 h in favor of *Pseudomonas* (50.8%), *Paenibacillus* (19.1%), and, to some extent, *Janthinobacterium* cells (11.7%).

For the lake community, *Aeromonas* was the most abundant genus in all samples and almost completely dominated the dispersed community under dry conditions (91.9% after 24 h and 95.6% after 48 h at -3.1 kPa) (Fig. 5). Under wet conditions, *Aeromonas* cells dispersed and colonized fast (79.1%, after 24 h) but appeared to experience increased competition from *Exiguobacterium* cells over time (43.3% and 37.5%, respectively, after 48 h).

The bacterial diversities, calculated using the Shannon diversity index (Fig. 6A and B; Fig. S3A and B), revealed that diversity was significantly affected by the matric potential in the 24-h soil samples ($P < 0.001$, analysis of variance [ANOVA] based on comparison of the differences between full plates and dispersed communities). The diversities under both the dry (-3.1 kPa) and moderately dry (-1.2 kPa) conditions were significantly different from those under the wet (-0.5 kPa) and very wet (0.0 kPa) conditions ($P < 0.05$ for all pairwise comparisons). The two dry (-3.1 and -1.2 kPa) and two wet (0.0 and -0.5 kPa) soil communities did not significantly differ from each other (Fig. S3B). At 48 h, the differences between the Shannon diversity indices under the four matric conditions were not significant ($P = 0.121$), in spite of a clear trend of decreasing diversity with drier conditions (Fig. 6B). The difference in diversities between the dispersed community and that recovered on the full plate could only be rigorously tested at 48 h, due to the significant effect of matric potential at 24 h, but the results showed that the dispersed soil community had a significantly lower diversity ($P = 0.001$) (Fig. 6B).

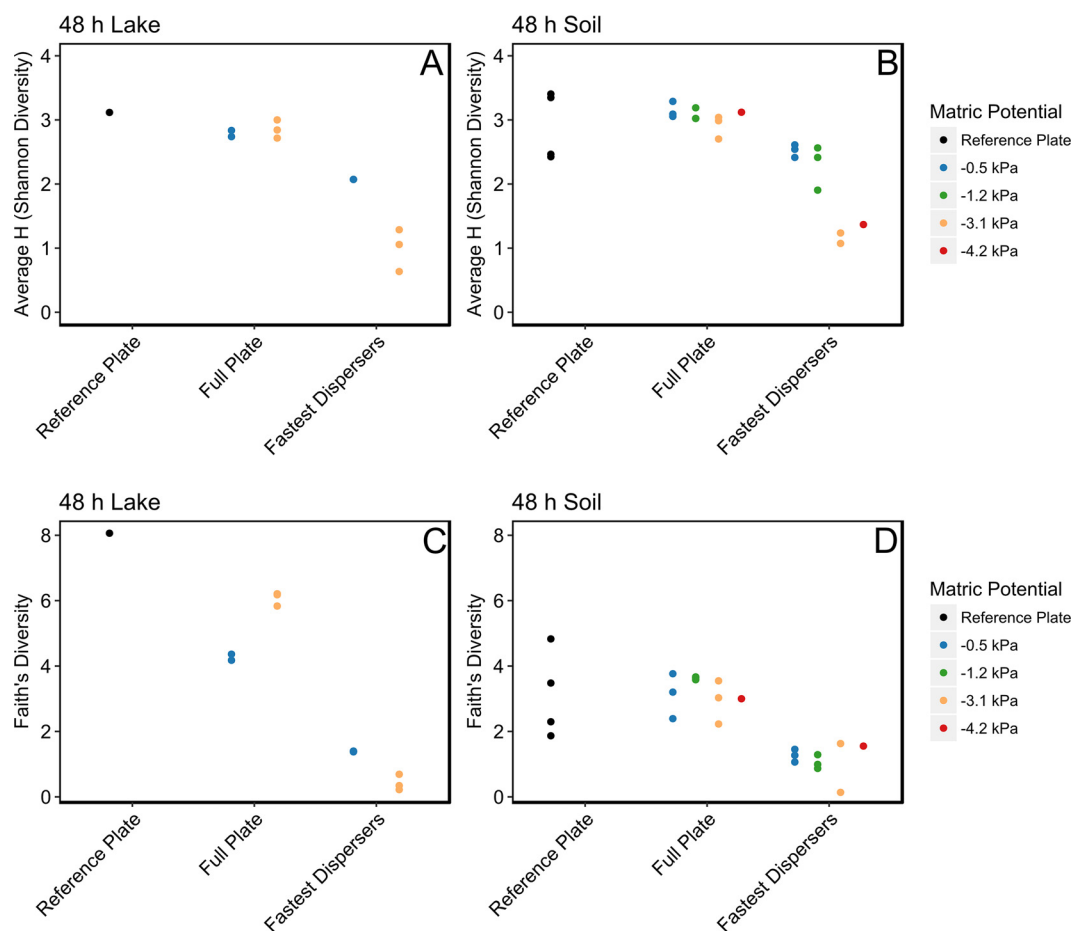


FIG 6 Estimates of alpha diversity (Shannon diversity index and Faith's phylogenetic diversity index) for communities derived from soil or from a lake after 48 h of incubation at different prescribed matric potentials. For each matric potential, the results for the total community recovered from the full agar plate (full plate) and the fastest-dispersed community are presented. A restricted-motility control (reference plate) is also included. Replicates are depicted as separate dots. The Faith's phylogenetic diversity indices reported are the averages of values obtained for 10 random rarefactions.

The lake data also indicated a trend for lower diversity in the dispersed community than in the full plate community at both 24 h and 48 h (Fig. 6A; Fig. S3A). As expected, the Shannon diversity values of the Nycodenz extract and the lake filtrate were much higher than those of the samples collected after cultivation on the agar plates (5.57 ± 1.45 [$n = 4$] and 3.82 [$n = 1$], respectively), clearly indicating a cultivation bias.

The phylogenetic diversity, calculated using Faith's phylogenetic diversity index (Fig. 6C and D; Fig. S3C and D), revealed consistently narrow phylogenetic diversities of the fastest dispersers at all matric potentials tested compared to the diversities of total communities of the full plate and the motility-restricted reference plate for both the soil and lake community.

A closer look at the phylogenetic distribution of members of the two dominant genera *Pseudomonas* and *Aeromonas* in soil and lake water, respectively, showed that the dispersed communities at 48 h consisted of multiple and diverse amplicon sequence variants (ASVs) (Fig. S4 and S5 and Tables S1 and S2). Notably, a search of the literature revealed that all the type strains with the closest sequence similarities to our ASVs possess the capability of motility, mainly by using flagella, except for one whose mode of motility is unknown (Tables S1 and S2). Neither for the soil community nor for the lake water community was there a clear separation of ASVs between matric conditions visible in the phylogenetic trees (Fig. S4 and S5).

A comparison of the *Pseudomonas* cells present in the total community of the full plate and the dispersed soil community at 48 h shows that only 11 of 44 ASVs were

solely present in the total community and, thus, did not disperse from the center of the ceramic disc (Fig. S6). This supports the general notion of pseudomonads as efficient dispersers. Furthermore, 9 of 44 ASVs were solely detected in the dispersed community. This is most likely because they were below the detection limit in the total community, as strains present in the dispersed community must also be present in the total community. Other evidence of large enrichments in the dispersed community can be found in the heatmap shown in Fig. 4, which shows that in addition to *Pseudomonas* cells, *Paenibacillus* (at matric potential below -3.1) and *Bacillus* (at -1.2 kPa) cells also notably increased their abundances in the dispersed community compared to their abundances in the total communities of the full plate and the motility-restricted reference plate. These results illustrate that there can be a large fitness gain associated with dispersal for a motile strain, i.e., going from being below the detection limit to potentially very high relative abundance far from the inoculation point. By moving ahead of the pack, such strains benefit from decreased competition for nutrients and maximize their growth.

DISCUSSION

Performance and limitations of the method. In this study, we developed a method for assessing the dispersal of natural bacterial communities under controlled hydration conditions. We achieved this by expanding on the porous surface model (PSM) already well established for single-strain motility studies (18, 35, 39), using agar plates to get an imprint of the colonization on the surface of the ceramic disc. The method proved effective in separating the dispersal of a motile flagellated *P. putida* strain from the limited spatial spreading of a nonmotile mutant, which stayed near the inoculation point in the center of the ceramic disc on the agar plate imprints (Fig. 1 and 2). It was also able to capture the effect of lowered matric potential, which resulted in a reduced dispersal rate of the motile strain and a cessation of all movement at -3.1 kPa, in agreement with previous studies (18, 34). In addition, the method was able to detect dispersal of *F. johnsoniae* gliding bacteria, indicating the potential for detection of other types of motility than swimming.

The possibilities for precise control of hydration conditions are one of the key points that separate this method from the few previous studies on community motility (27–29). Calculations coupled with recent measurements of the liquid film thickness on the surface of the ceramic disc in the PSM model (18, 34, 35) provide us with a unique platform to study the behavior of microbial communities on surfaces as they are affected in their microhabitats by water film thickness. While we only tested the effect of fixed hydration conditions in this study, exploring dynamic conditions like dry-wet cycles would be straightforward. Indeed, recent studies with a synthetic soil community on the PSM demonstrated a clear effect of such cycles on competition and coexistence (41).

We recognize that the results from using this method are biased by cultivation and are only valid for the fraction of bacteria able to grow under the selected growth conditions. However, we did find a high diversity of genera among the cultured community and that members of the most dominant genera, e.g., *Pseudomonas* and *Aeromonas*, were prominent parts of the original inocula. This indicates that, in spite of the existence of some cultivation bias, our method does provide information of relevance to the original communities.

In addition, nutrient supplementation is often necessary to detect dispersal (28), and one of the strengths of this setup is that it does allow for easy isolation of strains of interest, as we essentially already have them on agar plates. This has led to a culture collection of soil isolates able to disperse at -0.5 and -3.1 kPa for use in future studies (data not shown). A possible avenue to decrease cultivation bias is to optimize the medium. We currently use a medium with a relatively low substrate concentration (25% R2A and R2B) to avoid selection of only fast-growing bacteria, but this could be further improved by, e.g., using a soil extract medium (42). The results are also likely affected by the extraction methods used to obtain microbial inocula from the environment,

because extraction, and especially Nycodenz extraction (43, 44), affects the composition of the inoculum. However, this is not a limit to the method itself. The method would also be applicable when using intact environmental samples (for example, soil aggregates) placed in the center of the ceramic disc. Finally, while there are benefits of using the agar plate sampling method (low detection limit for cultivable bacteria), a possible improvement would be to recover the dispersed community for DNA extraction directly from the surface of the PSM. The recovery rate and detection limit would need to be evaluated carefully.

Dispersal of environmental communities under low-hydration conditions. When we applied the method to soil and lake water communities, the results extended previous pure-culture studies in confirming that dispersal rates decline as conditions become drier. However, surprisingly, relatively rapid dispersal of both communities was detected even under the lowest-hydration conditions (-3.1 kPa). After 48 h, members of both communities had reached the maximum possible distance of 25 to 41.3 mm (Fig. 3). It is unlikely that the detection of cells several centimeters away from the inoculation point could have been caused by simple colonial growth (i.e., cell division and shoving), because colony expansion by growth only is very slow (e.g., a diameter expansion rate of $17 \mu\text{m h}^{-1}$ for *P. putida* KT2440 at -3.6 kPa) (35). Therefore, this dispersal is likely facilitated by motility. This strong dispersal potential under low-hydration conditions was particularly surprising for the lake water community, because the selective value of such traits in the original habitat is not obvious.

A possible explanation for the rapid dispersal under conditions previously thought to be too dry could be a difference in cell size between the model strains and the bacteria in the environmental samples. As discussed in Dechesne et al. (18), the effective thickness of the liquid film on the surface of the ceramic disc is the limiting factor for flagellar motility. Pure-culture studies on the PSM using the motile strains *Pseudomonas protegens* strain CHA0 and *Pseudomonas putida* KT2440 report a threshold for swimming and dispersal at -2.0 kPa (18, 34, 35). At -2.0 kPa, the predicted effective liquid film thickness on the surface of the ceramic disc is less than $1.5 \mu\text{m}$, and it decreases to approximately $0.4 \mu\text{m}$ at -3.6 kPa, close to the shorter dimension of *P. putida* KT2440 rods (measured by others as $0.74 \mu\text{m}$ [rod shaped] [45] and as $0.6 \mu\text{m}$ by us under nutrient-rich conditions). Hence, motility becomes strongly limited in liquid films thinner than the cell diameter, due to exposing the cell surface to liquid-air interfaces, capillary pressure, and pinning forces (18, 35). As many bacteria from soil and aquatic environments are small, less than $0.4 \mu\text{m}$ in diameter or even able to pass through $0.2\text{-}\mu\text{m}$ filters (44, 46–48), it is possible for some of them to be able to actively disperse in the thinnest liquid films tested in this study. It should be noted, however, that as bacterial cell size can vary with the conditions, e.g., pseudomonads have been known to change both size and shape as a response to starvation or other chemical stressors (45, 49), the size of the bacteria used should be measured under the actual conditions imposed to confirm this theory.

Diversity of efficient dispersers. Our results show that diversity decreased in the dispersed communities compared to the diversities of the total communities in the soil and lake samples. This indicates that, within natural communities, there is a less-diverse subcommunity of bacteria with the potential for dispersal, which will most likely have important consequences for community composition, competition, and microbial succession. The study by Wolf et al. (28), which is most comparable to ours, identified the most abundant dispersers in their soil community as members of the family *Enterobacteriaceae* and the genera *Pseudomonas*, *Massilia*, and *Undibacterium*, with *Enterobacteriaceae* as the most dominant. Here, we also find members of both *Pseudomonas* and *Enterobacteriaceae* among the 20 most abundant ASVs in the dispersed soil community, and *Pseudomonas* is the most dominant genus among the dispersers. We also find *Paenibacillus* and *Cupriavidus* cells, which Wolf et al. (28) detected in low abundance, but along with *Enterobacteriaceae*, they are only present in our study under relatively wet conditions, with matric potentials of -1.2 kPa or lower. *Undibacterium*

cells were not present in either the initial community or the dispersed community, and *Massilia* cells were only detected in low numbers on one reference plate and in one of the Nycodenz extractions. The differences in the abundances and compositions of communities between the two studies are most likely caused by a combination of various factors, such as different initial communities in the inoculum, medium selection, and variation in hydration conditions. Nonetheless, it remains clear that pseudomonads play a key role in the two soil communities as early colonizers of unoccupied habitats and possibly gain a further advantage under relatively low-hydration conditions, where they dominate the community.

Potential modes of dispersal. Under dry conditions, *Pseudomonas* and *Aeromonas* cells dominated the dispersed soil and lake communities (Fig. 4 and 5). Many members of these two genera produce biosurfactants that have been shown to facilitate dispersal on surfaces (50–53) like leaves, an ability which has been hypothesized to increase fitness for members of *Pseudomonas* (54, 55). We speculate that biosurfactants also play a role in increasing the connectedness in the liquid film on surfaces. An important factor in our model system is the residual roughness of the ceramic surface, which although polished, can result in the fragmentation of the aqueous habitat as matrix potential decreases along with the liquid film thickness and, as a result, the topography of the surface becomes more apparent. Tecon and Or report that a rapid decrease in connectedness of the aqueous habitat was found at -2.0 to -5.0 kPa and that it influenced the motility of the flagellated bacteria they tested (34). Hence, biosurfactant production could be a strategy to overcome dispersal limitation under dry conditions for the two genera observed in our study. In addition, members of the order *Bacillales* (*Exiguobacterium*, *Bacillus*, and *Paenibacillus* spp.), which are frequently found in the dispersed lake and soil communities under wet conditions (Fig. 4 and 5), have also been found to produce biosurfactants, giving rise to speculation that the benefit of surfactant production for increased dispersal ability might not only be limited to dry conditions (53). While we did not look for biosurfactant production in this study, it would be straightforward to screen the isolates obtained for biosurfactant production in the future (56).

Alternative modes of surface motility apart from flagellum-powered swimming might play an increased role as conditions become dryer (7). Therefore, one of the strengths of the PSM for complex community studies is that it is not limited to investigating bacteria with swimming ability, as in the previous work by Grossart et al. (29) and Dennis et al. (27), but also enables studies of other modes, such as sliding, gliding, biosurfactant-aided movement, fungal highways (23), or even expansion by filamentous growth (32). The PSM could thus be instrumental in establishing which of these modes of motility are relevant on rough, unsaturated surfaces.

The pure-culture experiment with *F. johnsoniae* CJ1827 confirmed that gliding is possible and can provide a detectable dispersal advantage on the rough surface of the PSM. In the soil community experiments, *Flavobacterium* cells were detected in low abundances in the dispersed communities at -0.5 to -4.2 kPa (Fig. 4). Many members of this genus have been found to possess gliding motility (12, 57, 58), while flagellar motility in members of the family *Flavobacteriaceae* is almost unheard of (59), and recent isolates of the order *Flavobacteriales* from leaf surfaces have also been reported as biosurfactant producers (53). While the role of chemotaxis was not measured directly, it is possible that chemotactic organisms are enriched at the rim of the ceramic plate, where the substrate concentration is highest thanks to the low cell density. As many pseudomonads are known to possess chemosensory systems (60), this might contribute to their prevalence in the dispersed communities.

In theory, not all the strains we observe in our dispersed community have to possess the capability of active motility themselves; they might be nonmotile strains hitching a ride with their flagellated or gliding companions (21). The codispersal of multiple species unveils a much more complex picture of interactions that could be addressed by future studies employing the current PSM model system. A possible next step could

be to test the isolates obtained in this study to establish which are able to disperse autonomously versus relying on others.

Conclusion. A novel method to study motility at the community level was developed and tested on a soil and a lake microbial community. The results obtained suggest that within the motile fraction of a bacterial community, only a minority of the bacteria are able to disperse under relatively low-hydration conditions, previously thought too dry for flagellar motility. During dry periods, these highly efficient dispersers will gain a significant advantage with their ability to colonize new habitats ahead of the rest of the community. This highlights the need for increased focus on complex communities, rather than pure-culture studies, for the prediction of actual dispersal ability on solid surfaces like soil.

MATERIALS AND METHODS

Bacterial strains. The bacterial strain *Pseudomonas putida* KT2440 GFP, a GFP-tagged derivative of a motile bacterium initially isolated from rhizosphere soil (61), was used as a motile model strain for flagellar motility, and a previously created nonflagellated mutant, *P. putida* KT2440 DsRed Δ *flhM* (18), was used as a nonmotile model strain.

The bacterial strain *Flavobacterium johnsoniae* CJ1827 (37) was used as a model strain for gliding motility, and a nonmotile mutant, *F. johnsoniae* 2122 Δ *gldK* (38), was used as a nonmotile model strain. All strains were routinely maintained on agar plates. *P. putida* strains were grown on R2 agar (Fluka R2A; Sigma-Aldrich, St. Louis, MO) and *F. johnsoniae* strains on CYE agar (62) medium at 25°C.

Visualizing dispersal of nonfluorescent bacteria from environmental samples on the PSM. The porous surface model (PSM) has previously been described and used for observing the motility and growth of fluorescent strains after their inoculation at the center of a ceramic disc (diameter, 41.3 mm; thickness, 7.1 mm; maximum pore size, <1.5 μ m; 100 kPa bubbling pressure [Soilmoisture Equipment Corp., Santa Barbara, CA]) that simulates a soil surface under controlled hydration conditions (35). Imposing suction on the disc controls the thickness of the liquid film on the ceramic surface.

In this study, we have expanded the use of the PSM for environmental communities. As nonfluorescent cells are not detectable on the surface of the ceramic disc by standard microscopy, we trapped the bacteria from the PSM by pressing small agar plates on top of the ceramic disc. This allows visualizing the colonization on the ceramic disc by observing the growth on the corresponding agar plates (see Fig. S1 in the supplemental material).

The agar plates were obtained by pouring 6.3 ml of 25% R2A with 20 g agar liter⁻¹ into the lid of a small plastic petri dish (diameter, 40 mm; height, 12.5 mm [StarDish; Phoenix Biomedical Products, Mississauga, Canada]), filling it to the brim. To further flatten the surface of the agar, the sterile lid of a standard petri dish (diameter, 90 mm; height, 14.2 mm [VWR International, Søborg, Denmark]) was pressed on top of the small agar plate before it had completely solidified. After drying, the small agar plate was transferred into a large petri dish for storage. The PSM reservoirs were filled with 200 to 250 ml 25% R2B (Alpha Biosciences, Baltimore, MD) and autoclaved before use.

Preliminary tests with fluorescent strains revealed that pressing of agar plates on the ceramic discs provides a distorted image of the bacterial spatial pattern because cells are inevitably displaced along the contact plane. Therefore, we detected bacterial colonization in concentric annular sections of the PSM surface. By preparing agar plates with holes of diameters ranging from 11.5 mm to 15 mm, 20 mm, and 25 mm (Fig. S1), we could estimate dispersal by sequentially pressing these plates on the PSM, starting with that with the biggest hole and finishing with a full plate.

The holes were punched in the agar plates with a custom-made tool consisting of a Teflon handle (for safe handling during flame sterilization) fitted to brass tubes of various diameters (length, 12.5 cm; diameters, 11.5, 15, 20, and 25 mm) (Fig. S2). A printed template was placed under the agar plate to help center the holes. All plates were kept for a minimum of 48 h at room temperature before use on the PSM, to test for contamination.

Proof of concept with motile and nonmotile pure cultures. We tested the capability of the method of distinguishing the dispersal patterns of *P. putida* KT2440 GFP and *P. putida* KT2440 DsRed Δ *flhM* cells. The bacteria, cultivated on R2A plates, were suspended in a 0.9% NaCl solution and adjusted by optical density measurements at 600 nm to obtain a cell density of ca. 2,000 cells μ l⁻¹, as confirmed by plate counts. Before inoculation, the PSMs were elevated to -4.2 kPa (the length of the hanging water column is 40 cm and equals a suction of -4.2 kPa) for 20 min to drain excess fluid from the ceramic surface. The two bacterial suspensions were mixed in an equal ratio, and 0.5 μ l was inoculated in the center of the ceramic disc, where it was rapidly absorbed. The discs were then brought to matrix potentials (suction) of -0.5, -1.2, or -3.1 kPa (-5, -12, and -30 cm of water suction, respectively) and incubated at room temperature for 14, 24, or 40 h before being sampled by pressing the suite of agar plates onto the surface. Plates were incubated at 25°C for a 48-h growth period before being stored in the refrigerator at 4°C until observation by microscopy.

To test the applicability of the method for other types of motility, we tested the gliding bacterium *Flavobacterium johnsoniae* strain CJ1827 (37) and a nonmotile mutant, *F. johnsoniae* 2122 Δ *gldK* (38), on separate PSMs. Bacteria were streaked from CYE agar, grown in overnight cultures at 25°C in motility medium (MM) (63), and adjusted by optical density measurements at 600 nm to obtain a cell density of ca. 63,000 cells μ l⁻¹, as confirmed by plate counts, before inoculation of 1 μ l in the center of the ceramic

disc. The PSMs were kept at -0.5 kPa for 48 h of incubation at room temperature, using 25% R2B medium in the PSM reservoirs. The 25% R2A pressed plates were kept at 25°C for a 48-h period before growth was recorded.

Microscopy and imaging. The spatial patterns of *P. putida* KT2440 GFP and *P. putida* KT2440 DsRed Δ *flhM* on the PSM and on agar plates were determined with a Leica MZ16 FA epifluorescence stereomicroscope equipped for GFP and DsRed detection and fitted with a charge-coupled device (CCD) camera. Each plate was scored for the presence or absence of each strain. For documentation purposes, the entire surface of selected plates was imaged by sequentially capturing several fields of view, using a motorized stage piloted by Image Pro Plus (version 7.1; Media Cybernetics, Silver Spring, MD), and then assembling a tiled image using the same software. The GFP and DsRed images of each plate were captured separately and then combined into one image.

To document the presence of colonies on the plates independently of fluorescence, the plates were subsequently imaged using the camera of a Gel Doc XR system (Bio-Rad), operated in epi-white mode.

Dispersal potential of environmental communities. A soil sample was collected from the plow layer (5- to 15-cm depth) of a Danish agricultural field included in the Danish Pesticide Risk Assessment Program (PLAP) in March 2016 (Fårdrup, Sjælland). The soil is characterized by clay till, and further details can be found at <http://pesticidvarsling.dk/>. The soil was stored at 4°C . For each experiment, 25 g of sieved (2 mm) soil was taken by composite sampling, i.e., as small subsamples taken from the original soil sample and then mixed. The soil bacteria were extracted using Nycodenz density gradient centrifugation as described in reference 64, except for the final cell density determination, which was performed directly using a Thoma counting chamber. Cell density was adjusted to 0.5×10^6 to 1×10^6 cells μl^{-1} in 0.9% NaCl solution, and 10 μl was inoculated as 1- μl drops in the center of the ceramic discs. This inoculum corresponded to ca. 2,000 to 8,000 CFU on R2A plates. All plates used for the environmental communities were amended with 100 mg liter $^{-1}$ Delvodic (natamycin; DSM Food Specialties, Delft, The Netherlands) to inhibit fungal growth.

Lake water was sampled from the urban lake Sortedamssøen (Copenhagen) in September 2016. A 4-liter volume was collected from the surface water approximately 1.5 m from the shore. The water sample was filtered first through a 2- μm glass fiber prefilter (Merck Millipore, Tullagreen, Ireland) and then through 0.2- μm polycarbonate filters (GVS Filter Technology, Morecambe, United Kingdom) on a filtration manifold (DHI Lab Products; Hørsholm, Denmark). The filters were transferred into a 15-ml falcon tube with 2.5 ml 0.9% NaCl solution and vortexed for 45 s. The filters were removed, and the cell density was adjusted by Thoma count to 2×10^6 cells μl^{-1} . Twenty microliters of the suspension was inoculated as 1- μl drops, yielding 34,125 CFU per inoculum based on drop plate counts on R2A plates. Both the lake and soil inoculum were kept at 4°C overnight before inoculation on the ceramic discs. After inoculation, the discs were brought to matric potentials of -0.5 and -3.1 kPa and incubated at room temperature for 24 to 48 h before sampling.

After sampling by pressing the agar plate series on the PSMs at appropriate times, the plates were incubated for 72 h at 25°C . In addition to the presence/absence score used in the pure-culture studies, the coverages of bacterial growth on the individual agar plates were roughly estimated by eye using 4 categories, 1 to 25%, 26 to 50%, 51 to 75%, and 76 to 100% coverage.

After scoring, for each pressed-plate series, the plate with the fastest colonizers (bacteria present the furthest from center) and the full plate with the total cultivable community were chosen for amplicon sequencing. In addition to these, for each separate experiment, a reference plate was made by drop plating 10 μl of the inoculum onto the center of a small 25% R2A plate with 20 g agar liter $^{-1}$. This was meant as a motility-restricted control for the bacteria cultivable on the medium.

The bacteria were then washed from the agar plates by transferring the agar from the small petri dish into a standard-size petri dish with a flamed spatula, adding 2 ml 0.9% NaCl solution for 10 min, and then gently rubbing the surface of the agar with a sterile inoculation loop and collecting the bacterial suspension by pipetting into an Eppendorf tube. The procedure was repeated twice with 1.5 ml 0.9% NaCl, and the suspensions were collected. The Eppendorf tubes were centrifuged for 5 min at $7,500 \times g$ before pooling the bacteria into a single 1-ml sample suspension. The cell suspensions (plate wash) from the pressed plates, reference plates, Nycodenz extracts, lake filtrate, and leftover inoculums were all transferred to cryotubes and stored at -80°C .

DNA extraction and sequencing. DNA was extracted using the PowerLyzer PowerSoil kit (MoBio, Carlsbad, CA) by following the manufacturer's protocol with a few changes. An amount of 500 μl of the thawed plate wash was centrifuged for 5 min at $10,000 \times g$. The supernatant was removed and the pellet dissolved by adding 750 μl bead solution and vortexing. The suspensions were transferred to glass bead tubes, 60 μl of PowerLyzer PowerSoil kit solution C1 was added, and samples were placed in a Bead Beater for 5 min at 2,000 RPM. The manufacturer's protocol was then followed. DNA concentrations were measured on a Qubit 2.0 instrument (Life Technologies, Invitrogen, Carlsbad, CA) and samples stored at -80°C until sequencing.

The extracted DNA was PCR amplified using the universal primer set PRK341F (5'-CCTAYGGGRBGC ASCAG-3') and PRK806R (5'-GGACTACNNGGTATCTAAT-3'), which amplifies the V3-V4 hypervariable regions of the 16S rRNA genes (65). Purified PCR products (2×300 -bp reads) were sequenced on the Illumina MiSeq platform at the DTU Multi Assay Core Center (Lyngby, Denmark). All raw 16S rRNA gene amplicons were processed with the DADA2 pipeline (66) with default parameters. The sequences were classified based on the SILVA prokaryotic reference database, version 123 (67). A total of 3.8 million sequences passed the filtering steps, representing an average of 5.3×10^4 sequences per sample.

Shannon indices were computed in R software (version 3.3.1; R Core Team) using the `plot_richness` function in the `phyloseq` package (68). Samples were rarefied to an even depth (average of 10 iterations)

with the `rarefy_even_depth` function in the `phyloseq` package before calculating Faith's diversity with the `pd.query` function of the `PhyloMeasures` package (69). Heatmaps were plotted using the `amp_heatmap` function of the `ampvis` package (70), while `ggplot2` (71) and `ggtree` (72) were used for plots and phylogenetic trees, respectively.

Type strains were identified using EZBioCloud (www.ezbiocloud.net) (73), and the closest matches, along with sequences for common pseudomonads and aeromonads, were added to the trees for reference. For construction of phylogenetic trees with type strains, sequences were aligned with ClustalW in MEGA7 with the following parameters: pairwise alignment, gap open 1, extension 6.66; multiple alignment, gap open 15, extension 6.66. Sequences were trimmed to even lengths. The tree was constructed with the unweighted pair group method with arithmetic mean (UPGMA) function in the `phangorn` package (74).

Statistical analysis. The Mann-Whitney rank sum test in Sigmaplot 13 (Systat Software, Inc., San Jose, CA) was used for the dispersal profile data. One-way ANOVA based on comparison of the differences between full plates and dispersed communities was used for 24-h Shannon diversity indices. Kruskal-Wallis ANOVA on ranks (data were ranked due to unequal variance) and a paired *t* test were used for 48-h Shannon diversity indices. *P* values of <0.05 were considered significant.

Accession number(s). All sequencing data have been deposited as an NCBI BioProject under accession number [PRJNA400555](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA400555).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AEM.02857-17>.

SUPPLEMENTAL FILE 1, PDF file, 1.5 MB.

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REFERENCES

- Holt RD. 2009. IJEE soapbox: ecology and evolution as professions, and as liberal arts. *Isr J Ecol Evol* 55:307–313. <https://doi.org/10.1560/IJEE.55.4.307>.
- Reichenbach T, Mobilia M, Frey E. 2007. Mobility promotes and jeopardizes biodiversity in rock-paper-scissors games. *Nature* 448:1046–1049. <https://doi.org/10.1038/nature06095>.
- Bonte D, Dahirel M. 2017. Dispersal: a central and independent trait in life history. *Oikos* 126:472–479. <https://doi.org/10.1111/oik.03801>.
- Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, Ferrenberg S. 2013. Patterns and processes of microbial community assembly. *Microbiol Mol Biol Rev* 77:342–356. <https://doi.org/10.1128/MMBR.00051-12>.
- Bilton DT, Freeland JR, Okamura B. 2001. Dispersal in freshwater invertebrates. *Annu Rev Ecol Syst* 32:159–181. <https://doi.org/10.1146/annurev.ecolsys.32.081501.114016>.
- Dechesne A, Badawi N, Aamand J, Smets BF. 2014. Fine scale spatial variability of microbial pesticide degradation in soil: scales, controlling factors, and implications. *Front Microbiol* 5:1–14. <https://doi.org/10.3389/fmicb.2014.00667>.
- Or D, Smets BF, Wraith JM, Dechesne A, Friedman SP. 2007. Physical constraints affecting bacterial habitats and activity in unsaturated porous media: a review. *Adv Water Resour* 30:1505–1527. <https://doi.org/10.1016/j.advwatres.2006.05.025>.
- Henrichsen J. 1972. Bacterial surface translocation: a survey and a classification. *Bacteriol Rev* 36:478–503.
- Harshay R. 2003. Bacterial motility on a surface: many ways to a common goal. *Annu Rev Microbiol* 57:249–273. <https://doi.org/10.1146/annurev.micro.57.030502.091014>.
- Shrout JD. 2015. A fantastic voyage for sliding bacteria. *Trends Microbiol* 23:244–246. <https://doi.org/10.1016/j.tim.2015.03.001>.
- Hölscher T, Kovács ÁT. 2017. Sliding on the surface: bacterial spreading without an active motor. *Environ Microbiol* 19:2537–2545. <https://doi.org/10.1111/1462-2920.13741>.
- McBride MJ. 2001. Bacterial gliding motility: multiple mechanisms for cell movement over surfaces. *Annu Rev Microbiol* 55:49–75. <https://doi.org/10.1146/annurev.micro.55.1.49>.
- Snyder LAS, Loman NJ, Fütterer K, Pallen MJ. 2009. Bacterial flagellar diversity and evolution: seek simplicity and distrust it? *Trends Microbiol* 17:1–5. <https://doi.org/10.1016/j.tim.2008.10.002>.
- Liu R, Ochman H. 2007. Stepwise formation of the bacterial flagellar system. *Proc Natl Acad Sci U S A* 104:7116–7121. <https://doi.org/10.1073/pnas.0700266104>.
- Luciano J, Agrebi R, Le Gall AV, Wartel M, Fiegna F, Ducret A, Brochier-Armanet C, Mignot T. 2011. Emergence and modular evolution of a novel motility machinery in bacteria. *PLoS Genet* 7:e1002268. <https://doi.org/10.1371/journal.pgen.1002268>.
- Whitman WB, Coleman DC, Wiebe WJ. 1998. Prokaryotes: the unseen majority. *Proc Natl Acad Sci U S A* 95:6578–6583.
- Ben-Jacob E, Finkelshtein A, Ariel G, Ingham C. 2016. Multispecies swarms of social microorganisms as moving ecosystems. *Trends Microbiol* 24:257–269. <https://doi.org/10.1016/j.tim.2015.12.008>.
- Dechesne A, Wang G, Gulez G, Or D, Smets BF. 2010. Hydration-controlled bacterial motility and dispersal on surfaces. *Proc Natl Acad Sci U S A* 107:14369–14372. <https://doi.org/10.1073/pnas.1008392107>.
- Venturi V, Bertani I, Kerényi Á, Netotea S, Pongor S. 2010. Co-swarming and local collapse: quorum sensing conveys resilience to bacterial communities by localizing cheater mutants in *Pseudomonas aeruginosa*. *PLoS One* 5:e9998. <https://doi.org/10.1371/journal.pone.0009998>.
- Zhou J, Ma Q, Yi H, Wang L, Song H, Yuan YJ. 2011. Metabolome profiling reveals metabolic cooperation between *Bacillus megaterium* and *Ketogulonigenium vulgare* during induced swarm motility. *Appl Environ Microbiol* 77:7023–7030. <https://doi.org/10.1128/AEM.05123-11>.
- Finkelshtein A, Roth D, Ben Jacob E, Ingham CJ. 2015. Bacterial swarms recruit cargo bacteria to pave the way in toxic environments. *mBio* 6:e00074–15. <https://doi.org/10.1128/mBio.00074-15>.
- Ellegaard-Jensen L, Knudsen BE, Johansen A, Albers CN, Aamand J, Rosendahl S. 2014. Fungal-bacterial consortia increase diuron degradation in water-unsaturated systems. *Sci Total Environ* 466–467:699–705. <https://doi.org/10.1016/j.scitotenv.2013.07.095>.
- Warmink JA, Nazir R, Corten B, van Elsas JD. 2011. Hitchhikers on the fungal highway: the helper effect for bacterial migration via fungal hyphae. *Soil Biol Biochem* 43:760–765. <https://doi.org/10.1016/j.soilbio.2010.12.009>.
- Nazir R, Zhang M, de Boer W, van Elsas JD. 2012. The capacity to

- comigrate with *Lyophyllum* sp. strain Karsten through different soils is spread among several phylogenetic groups within the genus *Burkholderia*. *Soil Biol Biochem* 50:221–233. <https://doi.org/10.1016/j.soilbio.2012.03.015>.
25. Warmink JA, van Elsas JD. 2009. Migratory response of soil bacteria to *Lyophyllum* sp. strain Karsten in soil microcosms. *Appl Environ Microbiol* 75:2820–2830. <https://doi.org/10.1128/AEM.02110-08>.
 26. Brock DA, Read S, Bozhchenko A, Queller DC, Strassmann JE. 2013. Social amoeba farmers carry defensive symbionts to protect and privatize their crops. *Nat Commun* 4:2385. <https://doi.org/10.1038/ncomms3385>.
 27. Dennis PG, Seymour J, Kumbun K, Tyson GW. 2013. Diverse populations of lake water bacteria exhibit chemotaxis towards inorganic nutrients. *ISME J* 7:1661–1664. <https://doi.org/10.1038/ismej.2013.47>.
 28. Wolf AB, Rudnick M-BB, de Boer W, Kowalchuk GA. 2015. Early colonizers of unoccupied habitats represent a minority of the soil bacterial community. *FEMS Microbiol Ecol* 91:fiv024. <https://doi.org/10.1093/femsec/fiv024>.
 29. Grossart HP, Riemann L, Azam F. 2001. Bacterial motility in the sea and its ecological implications. *Aquat Microb Ecol* 25:247–258. <https://doi.org/10.3354/ame025247>.
 30. Fenchel T. 2001. Eppur si muove: many water column bacteria are motile. *Aquat Microb Ecol* 24:197–201. <https://doi.org/10.3354/ame024197>.
 31. Mitchell JG, Pearson L, Bonazinga A, Dillon S, Khouri H, Paxinos R. 1995. Long lag times and high velocities in the motility of natural assemblages of marine bacteria. *Appl Environ Microbiol* 61:877–882.
 32. Wolf AB, Vos M, de Boer W, Kowalchuk GA. 2013. Impact of matric potential and pore size distribution on growth dynamics of filamentous and non-filamentous soil bacteria. *PLoS One* 8:e83661. <https://doi.org/10.1371/journal.pone.0083661>.
 33. Wang G, Or D. 2010. Aqueous films limit bacterial cell motility and colony expansion on partially saturated rough surfaces. *Environ Microbiol* 12:1363–1373. <https://doi.org/10.1111/j.1462-2920.2010.02180.x>.
 34. Tecon R, Or D. 2016. Bacterial flagellar motility on hydrated rough surfaces controlled by aqueous film thickness and connectedness. *Sci Rep* 6:19409. <https://doi.org/10.1038/srep19409>.
 35. Dechesne A, Or D, Gulez G, Smets BF. 2008. The porous surface model, a novel experimental system for online quantitative observation of microbial processes under unsaturated conditions. *Appl Environ Microbiol* 74:5195–5200. <https://doi.org/10.1128/AEM.00313-08>.
 36. Jordan FL, Maier RM. 1999. Development of an agar lift-DNA/DNA hybridization technique for use in visualization of the spatial distribution of Eubacteria on soil surfaces. *J Microbiol Methods* 38:107–117. [https://doi.org/10.1016/S0167-7012\(99\)00082-2](https://doi.org/10.1016/S0167-7012(99)00082-2).
 37. Rhodes RG, Pucker HG, McBride MJ. 2011. Development and use of a gene deletion strategy for *Flavobacterium johnsoniae* to identify the redundant gliding motility genes *remF*, *remG*, *remH*, and *remI*. *J Bacteriol* 193:2418–2428. <https://doi.org/10.1128/JB.00117-11>.
 38. Shrivastava A, Johnston JJ, Van Baaren JM, McBride MJ. 2013. *Flavobacterium johnsoniae* *GldK*, *GldL*, *GldM*, and *SprA* are required for secretion of the cell surface gliding motility adhesins *SprB* and *RemA*. *J Bacteriol* 195:3201–3212. <https://doi.org/10.1128/JB.00333-13>.
 39. Dechesne A, Smets BF. 2012. *Pseudomonad* swarming motility is restricted to a narrow range of high matric water potentials. *Appl Environ Microbiol* 78:2936–2940. <https://doi.org/10.1128/AEM.06833-11>.
 40. Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J* 11:2639–2643. <https://doi.org/10.1038/ismej.2017.119>.
 41. Kleyer H, Tecon R, Or D. 2017. Hydration dynamics alters species composition of a synthetic soil bacterial community inhabiting unsaturated porous microcosms, abstr P-44, p 89. 14th Symp Bact Genet Ecol (BAGECO 14), Aberdeen, Great Britain, 4 to 8 June 2017.
 42. Liebeke M, Brözel VS, Hecker M, Lalk M. 2009. Chemical characterization of soil extract as growth media for the ecophysiological study of bacteria. *Appl Microbiol Biotechnol* 83:161–173. <https://doi.org/10.1007/s00253-009-1965-0>.
 43. Holmsgaard PN, Norman A, Hede SC, Poulsen PHB, Al-Soud WA, Hansen LH, Sørensen SJ. 2011. Bias in bacterial diversity as a result of Nycodenz extraction from bulk soil. *Soil Biol Biochem* 43:2152–2159. <https://doi.org/10.1016/j.soilbio.2011.06.019>.
 44. Portillo MC, Leff JW, Lauber CL, Fierer N. 2013. Cell size distributions of soil bacterial and archaeal taxa. *Appl Environ Microbiol* 79:7610–7617. <https://doi.org/10.1128/AEM.02710-13>.
 45. McTee MR, Gibbons SM, Feris K, Gordon NS, Gannon JE, Ramsey PW. 2013. Heavy metal tolerance genes alter cellular thermodynamics in *Pseudomonas putida* and river *Pseudomonas* spp. and influence amebal predation. *FEMS Microbiol Lett* 347:97–106. <https://doi.org/10.1111/1574-6968.12226>.
 46. Bakken LR, Olsen RA. 1987. The relationship between cell size and viability of soil bacteria. *Microb Ecol* 13:103–114. <https://doi.org/10.1007/BF02011247>.
 47. Wang Y, Hammes F, Boon N, Egli T. 2007. Quantification of the filterability of freshwater bacteria through 0.45, 0.22, and 0.1 μm pore size filters and shape-dependent enrichment of filterable bacterial communities. *Environ Sci Technol* 41:7080–7086. <https://doi.org/10.1021/es0707198>.
 48. Hahn MW. 2004. Broad diversity of viable bacteria in “sterile” (0.2 μm) filtered water. *Res Microbiol* 155:688–691. <https://doi.org/10.1016/j.resmic.2004.05.003>.
 49. Givskov M, Eberl L, Møller S, Poulsen LK, Molin S. 1994. Responses to nutrient starvation in *Pseudomonas putida* KT2442 analysis of general cross-protection, cell-shape, and macromolecular content. *J Bacteriol* 176:7–14. <https://doi.org/10.1128/JB.176.1.7-14.1994>.
 50. Raaijmakers JM, De Bruijn I, Nybroe O, Ongena M. 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiol Rev* 34:1037–1062. <https://doi.org/10.1111/j.1574-6976.2010.00221.x>.
 51. Ndlovu T, Khan S, Khan W. 2016. Distribution and diversity of biosurfactant-producing bacteria in a wastewater treatment plant. *Environ Sci Pollut Res* 23:9993–10004. <https://doi.org/10.1007/s11356-016-6249-5>.
 52. Ilori MO, Amobi CJ, Odocha AC. 2005. Factors affecting biosurfactant production by oil degrading *Aeromonas* spp. isolated from a tropical environment. *Chemosphere* 61:985–992. <https://doi.org/10.1016/j.chemosphere.2005.03.066>.
 53. Burch AY, Do PT, Sbodio A, Suslow TV, Lindow SE. 2016. High-level culturability of epiphytic bacteria and frequency of biosurfactant producers on leaves. *Appl Environ Microbiol* 82:5997–6009. <https://doi.org/10.1128/AEM.01751-16>.
 54. Lindow SE, Brandt MT. 2003. Microbiology of the phyllosphere. *Appl Environ Microbiol* 69:1875–1883. <https://doi.org/10.1128/AEM.69.4.1875-1883.2003>.
 55. Burch AY, Zeisler V, Yokota K, Schreiber L, Lindow SE. 2014. The hygroscopic biosurfactant syringafactin produced by *Pseudomonas syringae* enhances fitness on leaf surfaces during fluctuating humidity. *Environ Microbiol* 16:2086–2098. <https://doi.org/10.1111/1462-2920.12437>.
 56. Burch AY, Shimada BK, Browne PJ, Lindow SE. 2010. Novel high-throughput detection method to assess bacterial surfactant production. *Appl Environ Microbiol* 76:5363–5372. <https://doi.org/10.1128/AEM.00592-10>.
 57. Shrivastava A, Lele PP, Berg HC. 2015. A rotary motor drives *Flavobacterium* gliding. *Curr Biol* 25:338–341. <https://doi.org/10.1016/j.cub.2014.11.045>.
 58. Jarrell KF, McBride MJ. 2008. The surprisingly diverse ways that prokaryotes move. *Nat Rev Microbiol* 6:466–476. <https://doi.org/10.1038/nrmicro1900>.
 59. McBride MJ. 2014. The family *Flavobacteriaceae*, p 643–676. In Rosenberg E, DeLong E, Lory S, Stackebrandt E, Thompson F (ed), *The prokaryotes: other major lineages of Bacteria and the Archaea*, 4th ed. Springer, Heidelberg, Germany.
 60. Sampedro I, Parales RE, Krell T, Hill JE. 2015. *Pseudomonas* chemotaxis. *FEMS Microbiol Rev* 39:17–46. <https://doi.org/10.1111/1574-6976.12081>.
 61. Nakazawa T. 2002. Travels of a *Pseudomonas*, from Japan. *Environ Microbiol* 4:782–786. <https://doi.org/10.1046/j.1462-2920.2002.00310.x>.
 62. Lin D, McBride MJ. 1996. Development of techniques for the genetic manipulation of the gliding bacteria *Lysobacter enzymogenes* and *Lysobacter brunescens*. *Can J Microbiol* 42:896–902. <https://doi.org/10.1139/m96-115>.
 63. Liu J, McBride MJ, Subramaniam S. 2007. Cell surface filaments of the gliding bacterium *Flavobacterium johnsoniae* revealed by cryo-electron tomography. *J Bacteriol* 189:7503–7506. <https://doi.org/10.1128/JB.00957-07>.
 64. Klümper U, Dechesne A, Smets B. 2014. Protocol for evaluating the permissiveness of bacterial communities toward conjugal plasmids by quantification and isolation of transconjugants, p 275–288. In McGenity TJ, Timmis KN, Nogales B (ed), *Hydrocarbon and lipid microbiology protocols: genetic, genomic and system analyses of communities*. Springer Protocols Handbooks. Springer, Berlin, Germany. https://doi.org/10.1007/8623_2014_36.
 65. Yu Y, Lee C, Kim J, Hwang S. 2005. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time poly-

- merase chain reaction. *Biotechnol Bioeng* 89:670–679. <https://doi.org/10.1002/bit.20347>.
66. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 13:581–583. <https://doi.org/10.1038/nmeth.3869>.
 67. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* 35:7188–7196. <https://doi.org/10.1093/nar/gkm864>.
 68. McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. <https://doi.org/10.1371/journal.pone.0061217>.
 69. Tsirogiannis C, Sandel B. 2016. PhyloMeasures: a package for computing phylogenetic biodiversity measures and their statistical moments. *Ecography (Cop)* 39:709–714. <https://doi.org/10.1111/ecog.01814>.
 70. Albertsen M, Karst SM, Ziegler AS, Kirkegaard RH, Nielsen PH, Stokholm-Bjerregaard M. 2015. Back to basics: the influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. *PLoS One* 10:e0132783. <https://doi.org/10.1371/journal.pone.0132783>.
 71. Wickham H. 2009. ggplot2: elegant graphics for data analysis? Springer, New York, NY.
 72. Yu G, Smith DK, Zhu H, Guan Y, Lam TT-Y. 2017. ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol Evol* 8:28–36. <https://doi.org/10.1111/2041-210X.12628>.
 73. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. *Int J Syst Evol Microbiol* 67:1613–1617. <https://doi.org/10.1099/ijsem.0.001755>.
 74. Schliep KP. 2011. phangorn: phylogenetic analysis in R. *Bioinformatics* 27:592–593. <https://doi.org/10.1093/bioinformatics/btq706>.